



## NOTE

Pathology

# Basal cell adenocarcinoma in the gland of the third eyelid of a brown bear (*Ursus arctos*)

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**ABSTRACT.** The right third eyelid of an adult female brown bear (*Ursus arctos*) was swollen and removed. Histopathology revealed a tumor exhibiting proliferation with mild infiltration, consisting of multi-stratified glandular structures of the innermost laminal neoplastic cells and the basaloid neoplastic cells, and with eosinophilic thick basal lamina material around the glandular structures. Both types of neoplastic cells exhibited moderate anisokaryosis, and mitotic figures were observed in the basaloid neoplastic cells. The laminal neoplastic cells were cytokeratin (CK) 8/18-positive. In contrast, the basaloid neoplastic cells were CK14- and p63-positive, but  $\alpha$ -smooth muscle actin- and calponin-negative. The case described herein is the first report of basal cell adenocarcinoma in the gland of the third eyelid of a bear.

**KEY WORDS:** basal cell adenocarcinoma, third eyelid gland, *Ursus arctos*

In the World Health Organization classification of tumors of the gland of the third eyelid (GTE) in domestic animals, only adenocarcinoma is listed [20]. However, some variants have been reported in dogs [2, 4, 12]. Most reported cases of GTE in domestic animals have been in dogs [2, 3, 12], and a smaller number of cases have been reported in cats [3]. To the best of our knowledge, apart from dogs and cats, the only other species in which epithelial tumors of the GTE have been reported is horses [10, 16]. From the viewpoints of comparative oncological pathology, it is important to accumulate tumor cases of GTE in various animal species. Herein, we describe a case of basal cell adenocarcinoma (BCAC) in the third eyelid of a brown bear, *Ursus arctos*.

An adult female *U. arctos* housed in a bear zoo (Kitaakita Bear Garden, Kitaakita, Akita) exhibited conjunctival hyperemia in the right eye in February 2016. Cefalexin was administered orally, and lomefloxacin was also administered via eye drops in the right eye, but the conjunctival hyperemia did not improve. Since the third eyelid of the right eye was swollen (Fig. 1A), it was surgically excised under sedation in September 2016. The size of the tumor was 3.5 × 4.5 cm at the time of excision. To date, there has been no recurrence or metastatic signs.

For histopathological examination, sections from 10% neutral-buffered, formalin-fixed, paraffin wax-embedded samples were stained with hematoxylin and eosin, periodic acid-Schiff (PAS) and Alcian blue (pH 2.5). For immunohistochemical analysis, primary monoclonal antibodies against pan-cytokeratin (CK) (prediluted, clone AE1/AE3, Dako, Glostrup, Denmark.), vimentin (prediluted, clone V9, Dako) and CK8/18 (prediluted, clone CAM5.2, Becton-Dickinson, Franklin Lakes, NJ, U.S.A.) were used as markers for glandular cells, antibodies against CK14 (prediluted, clone LL002, Thermo Fisher Scientific, Waltham, MA, U.S.A.) and p63 (1 in 200 dilution, clone 4A4, Dako) were used as markers for basal and myoepithelial cells, and antibodies against  $\alpha$ -smooth muscle actin (SMA) (prediluted, clone 1A4, Dako) and calponin (1 in 1,000 dilution, clone hCP, Sigma-Aldrich, St. Louis, MO, U.S.A.) were used as markers for myoepithelial cells. All markers were labeled using an EnVision+System HRP-labeled polymer (Dako) and visualized via the Liquid DAB+Substrate Chromogen System (Dako). The antigen-retrieval treatments for all antibodies except CK8/18 involved immersion of the sections into an antigen-retrieval solution (Target Retrieval Solution, pH 6.0, Dako) and heating at 121°C for 5 min in an autoclave. Enzyme digestion using Ready-to-use Proteinase K (Dako) at room temperature for 10 min was employed for antigen-retrieval of CK8/18. For an internal positive control, a section of the normal gland from the third eyelid near the tumor was included.

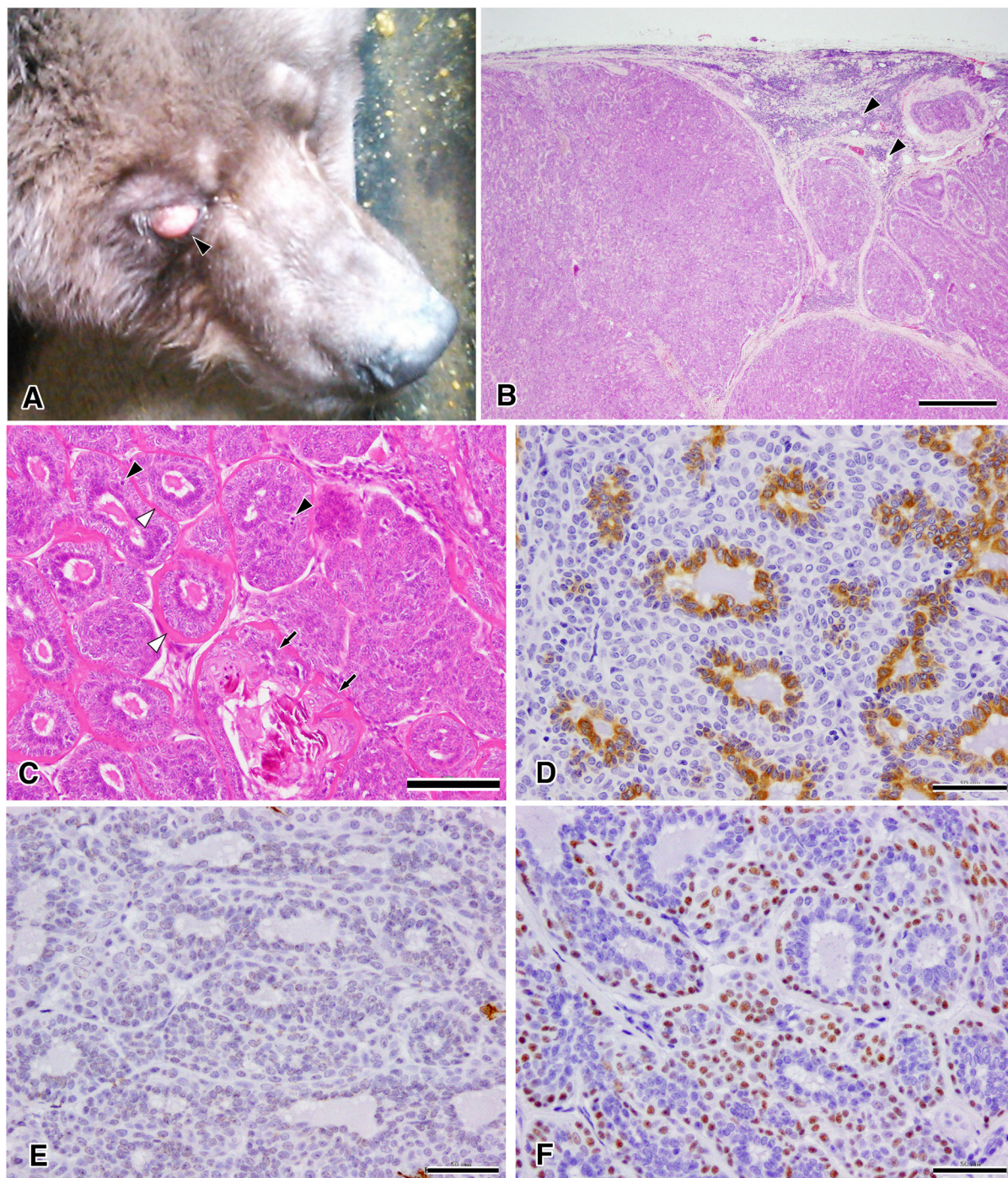
Microscopically, the tumor was relatively well-circumscribed, but proliferation with mild infiltration was evident, resulting in involvement of adjunct GTEs (Fig. 1B). The tumor was lobulated due to compartmentalization with connective tissue (Fig. 1B) and consisted of multi-stratified glandular structures of round cells. The innermost neoplastic cells of the glandular structures exhibited moderate amounts of eosinophilic cytoplasm and ovoid nuclei with one or a few small distinct nucleoli, and contained

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**Fig. 1.** Gross morphology, histopathology and immunohistochemistry of the third eyelid tumor in *U. arctos*. (A) Gross morphology. The swollen third eyelid of the right eye was protruded. (B) Histopathology of the tumor, low magnification. The tumor was compartmentalized with connective tissue, and exhibited proliferation and mild infiltration, resulting in the involvement of adjunct glands of the third eyelid (arrowheads). Lymphocytic inflammation was observed in the stroma. Hematoxylin and eosin stain, bar=1 mm. (C) Histopathology of the tumor, high magnification. The tumor consisted of multi-stratified glandular structures of round cells, prominently surrounded by eosinophilic thick basal lamina (white arrowheads). The innermost neoplastic cells of the glandular structures contained eosinophilic secretions in the lumen, and there were basaloid neoplastic cells surrounding the laminal epithelial neoplastic cells. Although there was no mitosis in the innermost neoplastic cells of the glandular structures, scattered mitotic figures were observed in the basaloid neoplastic cells (black arrowheads). A few squamous metaplastic foci were observed (arrows). Hematoxylin and eosin stain, bar=100  $\mu$ m. (D) Immunostaining of CK8/18. The innermost neoplastic cells of the glandular structures were positive, but the basaloid neoplastic cells were negative. Bar=50  $\mu$ m. (E) Immunostaining of SMA. Both types of neoplastic cells were negative. Bar=50  $\mu$ m. (F) Immunostaining of p63. The nuclei of the basaloid neoplastic cells were positive, but the innermost neoplastic cells were negative. Bar=50  $\mu$ m.



eosinophilic secretions in the lumen (Fig. 1C). The secretions were PAS- and Alcian blue-positive. The neoplastic cells surrounding the laminal epithelial neoplastic cells had less eosinophilic, moderately vacuolated or scant cytoplasm, and ovoid nuclei with small distinct nucleoli, similar to basal cells, and had a few scattered squamous metaplastic foci (Fig. 1C). In some parts of the tumor, abundant, prominently eosinophilic basal lamina material around the nests or glandular structures was observed (Fig. 1C). Lymphocytes and plasmacytes had infiltrated the stroma. Both neoplastic components revealed moderate anisokaryosis. Although there was no mitosis in the innermost laminal neoplastic cells of the glandular structures, scattered mitotic figures were observed in the basaloid neoplastic cells. There was no lymphatic or blood vessel invasion.

The cytoplasm of the innermost laminal neoplastic cells of the glandular structures was positive for pan-CK and CK8/18 (Fig. 1D), but negative for CK14, p63, calponin and SMA. The cytoplasm of the basaloid neoplastic cells surrounding the laminal neoplastic cells was positive for pan-CK and CK14, but negative for CK8/18, calponin and SMA (Fig. 1E). The nuclei of the outermost peripheral basaloid cells of the nests and glandular structures were strongly positive for p63, but this positivity became weaker near the centers of the nests and glandular structures (Fig. 1F). Both types of neoplastic cells were negative for vimentin.

Tumors of the GTE are rare in animals, and thus the histopathological features of such tumors have not been well characterized [20]. Some variants of epithelial tumors of the GTE, including BCACs in dogs, have only been reported recently [12]. Human basal cell adenoma and BCAC were reported in the salivary glands and the lacrimal glands [5–8, 13]. In humans, basal cell adenoma is characterized by a basaloid appearance of the tumor cells and absence of a myxochondroid stromal component characteristic of pleomorphic adenoma. BCAC is cytologically and histomorphologically similar to basal cell adenoma, but involves an infiltrative epithelial neoplasm with the potential for metastasis [9]. In the present case, distinct eosinophilic basal lamina material around the glandular structures of the tumor was observed. This was not definitively detected in a reported canine case [12]. Thus, the current case was more similar to human BCAC of the salivary gland than the previously reported cases in dogs [9, 12].

The neoplastic proliferation in the present case was dominated by round basaloid neoplastic cells positive for CK14 and p63, but negative for SMA and calponin, similar to canine cases [12]. In humans, the immunohistochemical features of salivary gland BCACs include positivity for not only CK and p63, but also for myoepithelial markers, such as SMA, CK14 and calponin [5, 7, 21]. However, neoplastic basaloid cells of lacrimal gland BCACs are negative for SMA [8]. Moreover, reported canine and feline salivary gland BCACs have typically been SMA-negative, except for one dog, which had tumor cells that were weakly positive for SMA [17]. The reduced SMA reactivity in BCACs might be caused by a loss of differentiation during neoplastic transformation, similar to that observed in canine mammary neoplasia [17, 19]. Thus, the immunoreactivities of myoepithelial markers in BCAC can change depending on the differentiation of neoplastic basal cells, and the basaloid neoplastic cells in the present case did not differentiate into myoepithelial cells.

Many adenocarcinomas in the GTE in dogs and cats have low-grade malignancy [4]. However, no follow-up data were available from canine BCAC in a previous report [12]. BCAC of the salivary gland in humans is considered to be a low-grade malignant tumor. They only metastasize occasionally and death is rare, although BCACs are locally destructive and often recur [5]. A dog and a cat with salivary gland BCAC in a previous report were humanely euthanized because of poor cosmetic prognoses, but there were no signs of metastasis [17]. In the present case, cellular atypia and invasive growth were also present, but neither recurrence nor metastasis has been observed to date and it was considered to be a low-grade malignant tumor.

There have been few reports of neoplastic diseases in the *U. arctos*, including mammary gland carcinoma [15, 18], chondrosarcoma [14], hepatocellular carcinoma [11] and lymphoma [1, 22]. However, to date, there have been no reports of tumors of GTE in the *Ursidae*. Therefore, the present case is the first report of basal cell adenocarcinoma in the gland of the third eyelid of a bear.

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